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Leukocyte Differentiation Mice (*Mus Musculus*) Infected with *Plasmodium Berghei* after Therapy With *Sargassum Duplicatum* Extract

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Abstract

Leukocytes are white blood cells produced by hematopoietic tissue that serves to help the body fight various infectious diseases as part of the immune system. Sargassum duplicatum contains steroid, alkaloid, phenol, flavonoid, saponin, and tannin compounds that can be immunomodulatory. The purpose of this research is to know the effect of methane extract of brown algae Sargassum duplicatum on the differentiation of leukocyte mice (Mus musculus) infected with Plasmodium berghei. Mice weighing 20-30 grams in Plasmodium berghei infection as much as 0.1 ml per head and left to the percentage of parasitemia reaches 1-5%. Then the mice (Mus musculus) were given brown algae methanol extract (Sargassum duplicatum) at doses 10, 100, and 200 mg / g BW for 4 consecutive days. From day 0 to 6 also made a blood smear to calculate the number of leukocytes. Data analysis will be done using ANOVA. The results showed that the administration of brown algae extracts of Sargassum duplicatum influenced in increasing the number of neutrophils, monocytes, and basophils and decreased the number of eosinophils and lymphocytes of mice infected with Plasmodium berghei to near normal range. Dose 200 mg/kg BB methanol extract of brown alga Sargassum duplicatum is a dose that can affect the number of neutrophils, eosinophils, lymphocytes, and monocytes of mice infected with Plasmodium berghei most closely to normal range.

Keywords: *Leukocyte Differentiation, Sargassum dupicatum, Immune*

Abstrak

Leukosit adalah sel darah putih yang diproduksi oleh jaringan hematopoietik yang berfungsi untuk membantu tubuh melawan berbagai penyakit infeksi sebagai bagian dari sistem kekebalan tubuh. Sargassum duplicatum mengandung senyawa steroid, alkaloid, fenol, flavonoid, saponin dan tannin yang dapat bersifat sebagai imunomodulator. tujuan dari penelitian ini adalah untuk mengetahui efek ekstrak methanol alga cokelat Sargassum duplicatum terhadap diferensiasi leukosit mencit (Mus musculus) terinfeksi Plasmodium berghei. Mencit dengan berat badan 20 – 30 gram di infeksi Plasmodium berghei sebanyak 0,1 ml per ekor dan dibiarkan sampai persen parasitemia mencapai 1-5%. Kemudian mencit (Mus musculus) diberi ekstrak methanol alga cokelat (Sargassum duplicatum) dengan dosis 10, 100 dan 200 mg/g BB selama 4 hari berturut-turut. dari hari ke 0 sampai ke 6 juga dibuat apusan darah untuk menghitung jumlah leukosit. Analisis data akan dilakukan dengan menggunakan ANOVA. Hasil penelitian menunjukkan bahwa pemberian ekstrak metanol alga cokelat Sargassum duplicatum berpengaruh dalam meningkatkan jumlah neutrophil, monosit dan basophil serta menurunkan jumlah eosinophil dan limfosit mencit terinfeksi Plasmodium berghei hingga mendekati kisaran normal. Dosis 200 mg/kg BB ekstrak metanol alga cokelat Sargassum duplicatum merupakan dosis yang mampu mempengaruhi jumlah neutrofil, eosinofil, limfosit, dan monosit mencit terinfeksi Plasmodium berghei yang paling mendekati kisaran normal.

Kata Kunci: *Diferensiasi Leukosit, Sargassum dupicatum, Sistem Imun,*

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INTRODUCTION

Indonesia is a country with a high malaria rate and it is estimated that 35% of the Indonesian population lives in malaria-endemic areas.[1,2] Eastern Indonesia regions such as Maluku, North Maluku, Papua, West Papua, and East Nusa Tenggara (NTT) are the provinces with the highest incidence of malaria.[3,4] For Every 1,000 residents in the area there are five malaria sufferers. Therefore, malaria is one of the infectious diseases that still requires attention in countermeasures.[5]

Treatment of malaria according to current WHO standards uses a combination of Artesunate and Piperaquin (dihydro artemisinin piperaquine / DHP or artarterakin).[6] Parasite resistance to commonly used antimalarials has continued to be reported from different parts of the world since 1973 to the present. To overcome the problem of resistance and reduce morbidity and mortality rates due to malaria disease WHO has instructed the cessation of all drug use as monotherapy and the application of treatment methods with Arterimycinin Combination Therapy (ACT).[7] This has led to the search for new compounds as antimalarial drugs and malaria inhibitors to continue to be carried out both from natural ingredients and synthetic products.

Sargassum duplicatum is a type of seaweed from the class *Phaeophyceae* that has antioxidant activity because it can inhibit fat peroxidation and free radical activity.[8] *Sargassum myriocystum* from the south coast of Tamil Nadu, India contains steroid compounds, alkaloids, phenols, flavonoids, saponins, and tannins.[9] The role of flavonoid compounds in inhibiting the growth of malaria parasites has been proven in some antimalarial medicinal plants.[1,2,7] Natural ingredient compounds such as flavonoids, lactones, lactones diterpenes, saponins, tannins, and terpenoids also act as immunomodulators.[10] According to Parlinaningrum et al.,[11] Immunomodulators are compounds that can increase the body's defense mechanisms both specifically and non-specifically.

Flavonoids work against lymphokines (Interferon γ) produced by T cells so that they will stimulate phagocytic cells to respond to phagocytosis and can spur lymphocyte proliferation, increase T cell count and increase secretion to IL-12. Flavonoids can increase the production of IL-2, one of the cytokines important for lymphocyte proliferation. Saponin compounds produce cytokines such as interleukin and interferons that play a role in the immunostimulant effect. Interleukin and interferons will react with antigens (foreign bodies) that enter the body.[12] Saponins in normal quantities act as immunostimulators, while in quantities exceeding the

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normal limits saponins will act as immunosuppressors.[13]

This research aimed to determine the differentiation of leukocytes of mice (*Mus musculus*) infected with *Plasmodium berghei* after being treated with brown algae methanol extract *Sargassum duplicatum.*

METHODOLOGY

Research Type

This research is an experimental laboratory.

Research Design

This research used a Complete Randomized Design (RAL) using 5 treatments and 3 repeats. The division of groups in this study according to Tanduwinata et al. is:[14]

1. K I: Group of mice that are only given equates (Negative control)
2. K II: Mice group infected with *Plasmodium berghei* but not given brown algae extract (Positive control).
3. K III: The group of mice infected with *Plasmodium berghei* and given brown algae extract at a dose of 10 mg / g BB.
4. K IV: Mice group infected with *Plasmodium berghei* and given brown algae extract at a dose of 100 mg/g BB.
5. K V: The group of mice infected with *Plasmodium berghei* and given a dose of brown algae extract 200 mg/g BB.

Research Tools and Materials

The tools used are measuring cups, Whatman 02 filter paper, a set of glassware, electronic scales, knives, blenders (smoothing tools), rotavapors, mice confinement containers, analytical balances, syringes, object glass containers, scratch slides, electron microscopy, tube centrifuges, hearing, sonde tools, volume pipettes, spatulas, test tubes, hand counters, satay grills, and digital cameras.

While the materials used are seaweed, absolute methanol 3 L, *Plasmodium berghei* culture Strain ANKA, male mice, aluminum foil, tissue, cotton, immersion oil, and detergent.

WORK PROCEDURE

Extraction

Sargassum duplicatum samples were taken from the waters of Liang Village, then taken to the laboratory, washed, and cut into small pieces after which they were dried in the laboratory. After drying, the sample is mashed with a blender to obtain simple from *Sargassum duplicatum*. It is further extracted by the maceration method using methanol solvent. The extraction results are concentrated with a rotary evaporator until a concentrated extract of *Sargassum duplicatum* is obtained.[15]

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Plasmodium berghei Infection in Donor Mice

Infection of donor mice with frozen deposits of *Plasmodium berghei* is carried out intraperitoneally. *Plasmodium berghei* as much as 200 µl into the body of the donor mice. Furthermore, daily observation of parasitemia until it reaches >20%, then surgery is performed to take blood from the heart of infected mice and put it into a blood tube (heparin bottle) and a percentage of parasitemia is obtained at 5%. Each mice were given 0.1 ml intraperitonial.[1]

In vivo anti-malarial activity testing

Mice infected with Plasmodium berghei were then observed in the degree of parasitemia by:

1. Blood is taken by cutting off the tip of the tail of the mice (± 1 mm).
2. Blood is dripped on the glass of the object. However, before a thin blood smear is made, the blood is allowed to widen left and right along the edges of the object's glass.
3. Furthermore, the object glass is rubbed towards the front along the surface of the preparation (preparation) of a thin layer of blood. And dried at room temperature.
4. After leaving to dry, the blood preparation is fixed with absolute methanol for 3 minutes.
5. Blood preparations are stained with a solution of Giemsa. The preparation is dripped completely with the solution and left for 45 minutes.
6. The preparations are washed under running water (angle 40 o) slowly and dried
7. The preparation is examined under a microscope after being dripped with immersion oil (magnification 10 x 100).
8. From the thin blood smear preparation examination, you will see the blood infected with malaria parasites.

If the level of parasitemia has reached 1%, antimalarial activity testing is carried out from the methanol extract of the brown algae *Sargassum duplicatum.* The administration of extracts in groups III, IV, and V mice was carried out for 4 consecutive days, and observed squeak parasitemia until day 6 to see the drug profile after extract administration was discontinued.[1]

Differential calculation of leukocytes

Every 100 leukocytes found are counted and grouped into each type of leukocyte, namely neutrophils, eosinophils, basophils, lymphocytes, and monocytes. The leukocyte calculation uses several fields of view along the smear that is shifted towards the center then shifted parallel to the edge of the smear and move to the edge back and so on until it reaches the number of leukocytes as much as 100.

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The relative value of each type of leukocyte found is expressed in units of percent.[16]

DATA ANALYSIS

The data from the calculation of the types of leukocytes will be analyzed in a one-way Analysis of Variance (ANOVA). If there is a noticeable difference, a Further test will be carried out using the Smallest Real Difference Test at a significant level of 0.05%.

RESULT AND DISCUSSION

Percentage of neutrophil count

The average percentage of neutrophils from day 0 (D0) to day 6 (D6) during the research can be seen in Table 1 below.

Table 1. The average percentage of neutrophil count

Day to - i	Percentage of Neutrophil count (%)					X ± SD
	Negative Control	Positive Control	Dosage 10 mg/g BB	Dosage 100 mg/g BB	Dosage 200 mg/g BB	
D0	20,33	12,67	14,50	16,83	14,83	**15,83±4,34[a]**
D1	19,5	10,67	13,17	12,50	14,67	**14,10±4,63[b]**
D2	18,67	8,83	10,3	11,33	14,50	**12,73±5,33[c]**
D3	20,00	7,50	9,67	10,67	15,00	**12,57±6,68[d]**
D4	19,50	6,17	9,17	9,83	15,67	**12,07±7,24[d]**
D5	19,17	5,17	8,83	9,33	17,00	**11,90±8,13[d]**
D6	19,50	5,50	7,33	8,67	17,33	**11,67±8,51[d]**
X ± SD	**19,52±2,03[a]**	**8,07±4,09[b]**	**10,29±3,76[c]**	**11,31±3,91[c]**	**15,57±2,94[d]**	**12,98±6,69**

Description: Superscripts with the same letter do not differ markedly (P > 0.05).
D0-D6: Days 0 to 6

The statistical results in Table 1 show that the average percentage of neutrophils in the negative control mice group was 19.52±2.03, and in the positive control group was 8.07±4.09. The average percentage of neutrophils in the group of mice infected with Plasmodium berghei treated with brown algae methanol extract *Sargassum duplicatum* dose 10 mg / g BB of 10.29±3.76, dose 100 mg / g BB of 11.31±3.91 and dose 200 mg / g BB of 15.57±2.94.

Based on the results of the two-way Analysis of Variance (ANOVA) using the SPSS 16.0 program, it shows that Fhitung> Ftabel, which means that the brown algae methanol extract of *Sargassum duplicatum* affects the average percentage of the number of neutrophils of mice infected with *Plasmodium berghei.*

The results of observations on the percentage of neutrophil counts showed that the average percentage of neutrophils in the group of mice infected with *Plasmodium berghei*

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treated with brown algae methanol extract *Sargassum duplicatum* doses of 10 mg / g BB, 100 mg / g BB and 200 mg / g BB increased when compared to positive controls (infected with *Plasmodium berghei* but not given brown algae methanol extract *Sargassum duplicatum* which tended to decrease (Table 1). The average percentage of neutrophils in the study was 11.12%. This value is close to the normal range proposed by Suhana,[17] which is 12-30%.

The decrease in the percentage of neutrophils in the positive group in this study was caused by *Plasmodium berghei* infection in mice, causing mice to suffer from malaria. In people with malaria shows a consistent effect on the activity of chemothatist, phagocytes, and microbicides of neutrophils, other changes that occur during inflammation in malaria include: decreased microvascular response to inflammatory mediators such as histamine and bradykinin, reduced protein leakage, and edema formation, reduced mast cell degranulation, impaired neutrophil adhesion to the endothelium and migration to the inflammatory site, production of reactive oxygen species and reduced release of cytokines and prostaglandins by neutrophils.[18]

Administration of brown algae methanol extract *Sargassum duplicatum* doses of 10 mg/g BB, 100 mg/g BB and 200 mg/g BB in this study may increase the percentage of neutrophil mice infected with *Plasmodium berghei.* This is thought to be because the brown algae *Sargassum duplicatum* contains secondary metabolite compounds such as flavonoids, tannins, and saponins.

Saponins can function as an immunostimulant that can boost the immune system.[19] Saponins produce cytokines such as interleukin and interferons that play a role in the immunostimulant effects. Interleukin and interferons will react with antigens (foreign bodies) that enter the body.[12] Saponins also can stimulate immune cells to increase the formation of antibodies so that they can act as immunostimulators.[20] Tannins play a role in increasing immunity with increased redox sensitivity in signaling systems involving the expression of certain genes. This change will further alter the function of some cells including immunostimulant function.[21]

Persentase jumlah Eosinofil

The average percentage of eosinophils from day 0 (D0) to day 6 (D6) of the research can be seen in Table 2 below.

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Table 2. The average percentage of the number of eosinophils

Day to - i	Percentage of the number of Eosinophils (%)					X ± SD
	Negative Control	Positive Control	Dosage 10 mg/g BB	Dosage 100 mg/g BB	Dosage 200 mg/g BB	
D0	1,50	1,67	1,83	2,33	2,50	**1,97±0,89[a]**
D1	0,50	3,00	3,00	2,50	1,83	**2,17±1,78[b]**
D2	1,00	4,00	3,00	3,67	2,00	**2,73±1,78[c]**
D3	1,00	4,33	3,50	4,50	2,33	**3,13±2,23[d]**
D4	0,67	5,67	3,50	4,00	2,83	**3,33±2,52[d]**
D5	1,83	6,33	4,33	4,17	0,50	**3,43±3,16[d]**
D6	1,17	6,17	5,50	4,50	0,67	**3,60±3,44d**
X ± SD	**1,10±1,09[a]**	**4,45±2,59[b]**	**3,52±1,74[c]**	**3,31±1,44[c]**	**1,81±1,44[d]**	**2,91±2,47**

Description: Superscripts with the same letter do not differ markedly (P > 0.05).
D0-D6: Days 0 to 6

Based on the results of statistical analysis in Table 2, it was shown that the average percentage of eosinophil numbers in the negative control group was 1.10±1.09, the positive control group was 4.45±2.59, in the mice group infected with *Plasmodium berghei* treated with brown algae methanol extract *Sargassum duplicatum* dose 10 mg/g BB was 3.52±1.74, dose 100 mg/g BB was 3.31±1.44 and dose 200 mg/g BB was 1.81±1.44.

The results of the two-way Analysis Of Variance (ANOVA) using the SPSS 16.0 program showed that F counted> F table, which means that the brown algae methanol extract *Sargassum duplicatum* affects the average percentage of the number of eosinophils of mice infected with *Plasmodium berghei.*

Eosinophils are white blood cells that belong to the granulosa. Eosinophils have a short time in blood circulation.[22] *Plasmodium berghei* infection in this study led to a decrease in the percentage of eosinophils. This is thought to be because eosinophils release proteins, cytokinins, and chemokines which result in an inflammatory reaction due to toxic compounds in the form of GPI contained in the Plasmodium membrane, then phagocytize these compounds.[23] Eosinophils that have phagocytes tend to die so that the eosinophils in the leukocytes are reduced. Eosinophil cells have limited phagocytic activity.[24]

The percentage of eosinophils that tended to be higher on positive controls in this study was assumed to be a response to the presence of malaria parasites. This is to the statement of Andiarsa et al.,[25] which states that eosinophils play a role in the body's immune process against the presence of parasitic infections such as worms, protozoa, and others. There is a meaningful association between the presence of eosinophils in a large percentage of the presence of malaria

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parasites (*Plasmodium* spp) but there is no meaningful relationship between eosinophils in a large percentage of the number of parasites present in malaria sufferers.[16]

The decrease in the percentage of eosinophils after being given *Sargassum duplicatum* brown algae methanol extract in this research was due to chemical compounds contained in it such as flavonoids. Flavonoids can improve the work of the immune system, saponins can stimulate immune cells to increase the formation of antibodies and tannins play a role in immunostimulant function.[26,27]

Percentage of Lymphocyte count

The average percentage of lymphocytes from day 0 (D0) to day 6 (D6) during the research can be seen in Table 3 below.

Table 3. The average percentage of the number of lymphocytes

Day to - i	Percentage of Lymphocyte count (%)					X ± SD
	Negative Control	Positive Control	Dosage 10 mg/g BB	Dosage 100 mg/g BB	Dosage 200 mg/g BB	
D0	74,50	81,17	80,50	78,50	80,50	**79,03±3,92^{a}**
D1	78,17	83,17	82,33	81,83	79,67	**81,03±3,06^{b}**
D2	77,33	85,33	84,67	81,83	81,17	**82,07±4,34^{c}**
D3	76,33	85,67	85,67	83,00	80,67	**82,27±5,23^{c}**
D4	76,50	87,33	86,33	83,33	81,33	**82,97±5,90^{c}**
D5	75,50	88,33	86,33	84,50	81,83	**83,30±6,79^{c}**
D6	77,50	88,67	87,33	85,00	82,83	**84,27±5,94^{c}**
X ± SD	**76,55±2,49^{a}**	**85,67±3,84^{b}**	**84,74±3,70^{c}**	**82,57±3,15^{d}**	**81,14±2,14^{e}**	**82,13±5,51**

Description: Superscripts with the same letter do not differ markedly (P > 0.05).
D0-D6: Days 0 to 6

From Table 3, it can be seen that the average percentage of lymphocytes in the negative control group was 76.55±2.49, in the positive control group was 85.67±3.84, in the mice group infected with *Plasmodium berghei* treated with brown algae methanol extract *Sargassum duplicatum* dose 10 mg / g BB of 84.74±3.70, dose 100 mg / g BB of 82.57±3.15 and dose 200 mg / g BB of 81.14±2.14,

The results of a two-way Analysis Of Variance (ANOVA) using the SPSS 16.0 program showed that F counted> F table, which means that the brown algae methanol extract *Sargassum duplicatum* affects the average percentage of the number of mice lymphocytes infected with *Plasmodium berghei.*

Plasmodium berghei infection in this research caused an increase in the presentation of the percentage of the number of mice lymphocytes. This is

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due to the presence of malaria infection in mice, so the body produces more lymphocytes. Lymphocytes are the type of leukocytes that work most specifically against foreign bodies entering the body, so the percentage of their number can increase when infection occurs.[28]

The mechanism of increasing the percentage of lymphocytes in this research is suspected to be through the leptin and IL-6 pathways produced by adipose tissue in diabetes mellitus patients so that the production and differentiation of lymphocytes increases. One of the most important effects of IL-6 is that it initiates the formation of an acute-phase response. In this acute-phase response, proteins will be formed that are synthesized and secreted by hepar, these proteins are known as acute-phase proteins. One of the acute-phase proteins is the C-reactive protein (CRP). CRP can activate the complement cascade by binding to C1q, which is the first component of complement activation on the classical pathway. In addition, IL-6 functions in immunity both innate immunity and adaptive immunity. This cytokine has various functions, in innate immunity can stimulate the synthesis of acute phase proteins by hepatocytes, thus playing a role in systemic inflammatory effects. IL-6 is also able to stimulate the production of neutrophils from progenitor cells residing in the bone marrow, usually with Colony Stimulating Factor (CSF).[1]

In adaptive immunity, IL-6 stimulates the growth of differentiated B cells and will later produce antibodies. The lymphocytes (Thelpers) play a major role in regulating and developing the immune response. Functionally Th lymphocytes are divided into two sub-classes namely Th1 and Th2, this division affects the secretion of cytokines it produces. The cytokines produced by Th1, namely IFN-γ can cause direct inflammatory reactions by stimulating macrophages. Th1 in small doses also stimulates B lymphocytes to produce Ig G, but in high levels for example severe infections will suppress the function of B lymphocytes, so there is often suppression of Ig G production.[1]

Administration of brown algae methanol extract *Sargassum duplicatum* doses of 10 mg / g BB, 100 mg / g BB and 200 mg / g BB in the reseacrh were able to reduce the presentation of the percentage of the number of mice lymphocytes (Table 3). This can be seen by the low presentation of the percentage of lymphocyte count in the group of mice infected with *Plasmodium berghei* treated with brown algae methanol extract *Sargassum duplicatum* when compared to the percentage of lymphocyte count in the positive group.

The decrease in the presentation of the percentage of lymphocytes in this

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research was due to flavonoid compounds, tannins, and saponins in the brown algae *Sargassum duplicatum* which is antimalarial. In addition to acting as antimalarials, these three compounds also act as immunomodulators that can restore and improve the immune system whose function is disrupted or suppress excessive functioning, so that the percentage of lymphocytes in the treatment group is lower than the positive control group in the first 4 days after extract administration, even the treatment group doses of 100 mg / g BB and 200mg / g BB are decreasing towards the normal range on days 5 and 6.

Percentage of the number of Monocytes

The average percentage of the number of monocytes from day 0 (D0) to day 6 (D6) during the research can be seen in Table 2 below.

Table 4. The average percentage of the number of monocytes

Day to - i	Percentage of the number of Monocytes (%)					X ± SD
	Negative Control	Positive Control	Dosage 10 mg/g BB	Dosage 100 mg/g BB	Dosage 200 mg/g BB	
D0	3,67	4,00	2,33	2,33	2,67	**2,87±1,59[a]**
D1	2,17	2,33	1,67	3,00	2,67	**2,37±1,58[a]**
D2	3,00	1,33	1,67	2,67	2,33	**2,23±1,40[b]**
D3	2,33	1,33	1,33	1,67	1,67	**1,67±1,16[c]**
D4	1,83	0,00	1,00	1,33	1,33	**1,03±1,49[d]**
D5	3,17	0,00	0,33	0,33	1,00	**0,73±1,96[e]**
D6	1,50	0,00	0,00	0,00	1,00	**0,30±,94[f]**
X ± SD	**2,52±2,52[a]**	**1,28±1,38[b]**	**1,14±1,14[b]**	**1,62±1,64[b]**	**1,81±1,31[b]**	**1,60±1,90**

Description: Superscripts with the same letter do not differ markedly (P > 0.05).
D0-D6: Days 0 to 6

The average percentage of monocytes in the negative group was 2.52±2.52, in the positive group was 1.38±1.38, in the mice group infected with *Plasmodium berghei* treated with brown algae methanol extract *Sargassum duplicatum* dose 10 mg / g BB of 1.14±1.14, dose 100 mg / g BB of 1.64±1.64 and 200 mg / g BB of 1.81±1.31. Based on the results of the two-way Analysis of Variance (ANOVA) using the SPSS 16.0 program, it shows that F calculates> F table, which means that the brown algae methanol extract *Sargassum duplicatum* affects the average percentage of the number of mice monocytes infected with *Plasmodium berghei*.

Monocytes are formed in the bone marrow, enter the circulation in imitation, and undergo a maturation process into macrophages after entering the tissues.[29] When the

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apoptosis program has been completed on a cell, it will leave a dead cell piece called the apoptosis body that will be recognized by the macrophage cells and eaten (engulfed).[30]

The average percentage of mice monocytes in the positive group during the observation day ranged from 0 – 2.67%. The percentage of the number of normal mice monocytes ranges from 1-12%. While the average percentage of monocytes of the mice group infected with *Plasmodium berghei* treated with brown algae methanol extract *Sargassum duplicatum* doses of 10 mg/g BB, 100 mg/g BB and 200 mg/g BB tended to be higher when compared to the positive control group. This proves that monocytes can kill malaria parasites in the blood. Monocytes also function to protect the body against invading organisms, especially with phagocytosis.[31] Phagocytic activity of monocytes depends on the material being phagocyted, but it is suspected that the flavonoid and saponin content in the brown algae methanol extract *Sargassum duplicatum* helps the work of monocytes thereby increasing the percentage of the number of monocytes in the group of mice infected with *Plasmodium berghei* treated with brown algae methanol extract *Sargassum duplicatum* compared to the control group positive.

Percentage of Basophil count

The average percentage of basophils from day 0 (D0) to day 6 (D6) during the research can be seen in Table 2 below.

Table 5. The average percentage of basophils

Day to -i	Percentage of Basophil count (%)					X ± SD
	Negative Control	Positive Control	Dosage 10 mg/g BB	Dosage 100 mg/g BB	Dosage 200 mg/g BB	
D0	0,00	0,50	1,00	0,33	0,00	**0,37±1,03**
D1	0,00	0,33	0,83	0,33	0,33	**0,37±,71**
D2	0,50	0,33	0,67	0,67	0,00	**0,40±,81**
D3	0,00	0,83	0,17	033	0,33	**0,37±,89**
D4	0,17	0,00	0,33	0,67	1,00	**0,27±,75**
D5	0,00	0,00	0,00	1,67	1,67	**0,47±1,40**
D6	0,33	0,00	0,33	1,67	2,33	**0,53±1,35**
X ± SD	**0,14±,507[a]**	**0,29±,793[b]**	**0,48±,96[c]**	**0,81±1,52[d]**	**0,81±,57[d]**	**0,40±1,00**

Description: Superscripts with the same letter do not differ markedly (P > 0.05).
D0-D6: Days 0 to 6

Based on the results in Table 5, it can be seen that the average percentage of basophils in the negative control group was 0.14±.507, the positive

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control group was 0.29±.793, in the mice group infected with *Plasmodium berghei* treated with brown algae methanol extract *Sargassum duplicatum* dose 10 mg/g BB was 0.48±.961, the dose was 100 mg/g BB was 0.81±1.523 and 200 mg/g BB was 0.81±, 569. Based on the results of the two-way Analysis of Variance (ANOVA) using the SPSS 16.0 program shows that F counts> F table, which means that the brown algae methanol extract *Sargassum duplicatum* affects the average percentage of the number of mice basophils infected with *Plasmodium berghei.*

The percentage of basophil counts found in this research was very small and low in the negative control group, positive control, and mice infected with *Plasmodium berghei* treated with brown algae methanol extract *Sargassum duplicatum* (Table 5). Basophils play a major role in various allergic and wound closure processes and basophils play less of a role in the presence of parasites.[32]

The percentage of basophils in blood circulation is relatively small (0.07 %), so in this research, no basophil cells were found. The basophil cells contained the substance heparin (anticoagulant). This heparin is released in the area of inflammation to prevent the onset of clots as well as static blood and lymph, so basophil cells are thought to be precursors to mast cells. Basophilia is an increase in the percentage of the number of basophils in circulation. Basophilia in domestic animals can occur due to hypothyroidism or estrogen injections. A decrease in the percentage of basophil cell count in the blood circulation or basopenia can occur due to corticosteroid injections at the stalemate stage.[33]

Although the percentage of basophils found in the research was very small, when viewed in Table 5, the percentage of basophil counts in the group of mice infected with *Plasmodium berghei* treated with brown algae methanol extract *Sargassum duplicatum* doses of 10 mg/g BB, 100 mg/g BB and 200 mg/g BB were slightly higher when compared to the positive control group. This indicates that the brown algae methanol extract *Sargassum duplicatum* has a hypersensitivity effect.

In general, the differential of mice leukocyte cells in this research increased the percentage of the number of neutrophils, monocytes, and basophils and decreased the percentage of the number of eosinophils and lymphocytes. The type of leukocyte that plays a very important role in leukocyte differentiation is neutrophil cells so a smaller percentage of neutrophil cells are found in mice suffering from malaria. The decrease in the percentage of neutrophils is due to a decrease in the viability of neutrophils in the cell circulation, a decrease in the percentage of the number of neutrophils in the bone

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marrow, and the percentage of the number of ineffective neutrophils in acute infectious conditions, septicemia, toxemia, radiation and the subsidence of an infection.

CONCLUSION

The administration of brown algae methanol extract *Sargassum duplicatum* has an effect in increasing the number of neutrophils, monocytes, and basophils and reducing the number of eosinophils and lymphocytes of mice infected with *Plasmodium berghei* to close to the normal range. A dose of 200 mg/kg BB of brown algae methanol extract *Sargassum duplicatum* is a dose capable of affecting the number of neutrophils, eosinophils, lymphocytes, and monocytes of mice infected with *Plasmodium berghei* which is closest to the normal range.

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